

CLINICAL AND LABORATORY PROFILES OF BLOOD DONORS WITH ERYTHROCYTOSIS

BY

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6. LIST OF ABBREVIATIONS

ASO	Allele specific oligonucleotide
BPG	Biphosphoglycerate
CO	Carbon monoxide
COHb	Carboxyhaemoglobin
CRP	C- reactive protein
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EEC	Endogenous erythroid colony
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin
EPOR	Erythropoietin receptor
ET	Essential thrombocythaemia
FBC	Full blood count
FBP	Full blood picture
G	Guanine
G-CSF	Granulocytes colony stimulating factors
GM	Granulocytes and monocytes/macrophages
Hb	Haemoglobin
HCT	Haematocrit
HPLC	High Performance Liquid Chromatography
HSAJB	Hospital Sultanah Aminah Johor Bahru
HUSM	Hospital Universiti Sains Malaysia

IE	Idiopathic erythrocytosis
IL	Interleukin
IMF	Idiopathic Myelofibrosis
JAK	Janus Kinase
JH	JAK homology
LFT	Liver function test
MCH	Mean Corpuscular Haemoglobin
MCV	Mean Corpuscular Volume
Meg-E	Megakaryocytes-Erythrocytes
MLR	Multiple linear regression
MPN	Myeloproliferative neoplasm
MREC	Medical Research and Ethics Committee
O ₂	Oxygen
PCR	Polymerase chain reaction
PCV	Packed cell volume
PHD	Prolyl hydroxylase
PKD	Polycystic kidney disease
PTE	Post renal transplant erythrocytosis
PV	Polycythaemia Vera
RBC	Red Blood Cell
RCM	Red cell Mass
RFT	Renal function test
SD	Standard deviation
SEAO	South East Asian ovalocytosis

SLR	Simple linear regression
SPSS	Statistical Packages for Social Sciences
STAT	Signal Transducers and Activators of Transcription
T	Thymine
TRBC	Total red blood cell
TWBC	Total white blood cell
VHL	Von Hippel Lindau
WBC	White blood cell count

7. ABSTRAK

PROFIL KLINIKAL DAN MAKMAL BAGI PENDERMA DARAH DENGAN ERYTHROSITOSIS.

Dalam perubatan transfusi, setiap penderma darah adalah diwajibkan untuk menjalani ujian pengesanan terhadap nilai hemoglobin (Hb) mereka terlebih dahulu sebelum prosedur pendermaan darah. Mengikut amalan semasa, penderma darah yang didapati mempunyai nilai Hb yang tinggi turut dikecualikan daripada pendermaan darah. Tiada perhatian khusus diberikan terhadap penderma sedemikian walaupun umum mengetahui bahawa nilai Hb yang tinggi mungkin menunjukkan adanya patologi yang tersembunyi. Oleh sebab itu, tujuan kajian ini adalah untuk menilai kemungkinan etiologi yang berkaitan dengan erythrositosis dikalangan penderma darah.

Satu kajian silang rentas telah dijalankan di Hospital Sultanah Aminah Johor Bahru (HSAJB) dan Hospital Universiti Sains Malaysia Kubang Kerian (HUSM) dalam tempoh sembilan (9) bulan dimana melibatkan penderma darah dengan pra-pendermaan nilai Hb yang tinggi. Sebanyak 175 sampel penderma telah dikumpulkan dan dianalisa untuk ujian hematologi, analisa Hb, ferritin, ujian biokimia serta kajian molekular bagi mengesan mutasi JAK2 V617F. Semua data dianalisa dengan menggunakan perisian SPSS versi 23.0.

Prevalens penderma darah yg didapati mempunyai nilai Hb yang tinggi adalah 7.8% (n=175). Di antara 175 orang penderma darah, 103 penderma didapati mempunyai erythrositosis relatif, dimana kebanyakannya mungkin disebabkan oleh pengecutan isipadu

plasma berpunca daripada dehidrasi. Sebaliknya, tujuh puluh dua (72) penderma lain adalah didapati mempunyai erythrositosis mutlak. Sebanyak empat puluh sembilan (49) penderma didapati mempunyai latar belakang sebagai perokok kronik. Kami mendapati bahawa adanya hubungan antara status merokok dan talasemia/ hemoglobinopati dengan erythrositosis di kalangan penderma darah. Kami juga mendapati hubungan yang positif antara bilangan pendermaan darah dengan tahap erythrositosis ($r=0.201$). Semakin tinggi bilangan pendermaan darah, semakin tinggi nilai Hb yang akan didapati pada penderma tersebut.

Kesimpulannya, nilai Hb yang tinggi dikalangan penderma darah menunjukkan etiologi patologi yang tersembunyi. Beberapa penderma didapati mempunyai masalah perubatan seperti talasemia/ hemoglobinopati dan kekurangan zat besi. Kami juga ingin mencadangkan bahawa penderma darah dengan nilai Hb yang tinggi perlu disiasat dengan lebih teliti untuk memastikan penderma ini tidak mengidapi sebarang penyakit serius yang berkaitan dengan erythrositosis.

8. ABSTRACT

CLINICAL AND LABORATORY PROFILES OF BLOOD DONORS WITH ERYTHROCYTOSIS

In blood banking setting, every blood donors are mandatory to have their haemoglobin (Hb) values tested before blood donation procedure. In current practice, donors with high Hb will be deferred from blood donation. Little attention is given to blood donors with high Hb although it is known that high Hb may indicate an underlying hidden pathological condition. Therefore, the aim of this study is to evaluate the possible underlying aetiologies associated with erythrocytosis in blood donors.

A pilot cross sectional study was conducted in Hospital Sultanah Aminah Johor Bahru (HSAJB) and Hospital Universiti Sains Malaysia Kubang Kerian (HUSM) over nine-month period involving blood donors with high pre-donation Hb. A total of 175 samples were collected and analysed for haematological tests, Hb analysis, serum ferritin, biochemical tests and molecular study for the detection of JAK2 V617F mutation. All of the data were analysed using SPSS software version 23.0.

The prevalence of blood donors with erythrocytosis was 7.8% (n=175). Among 175 donors, 103 donors were found to have relative erythrocytosis, which mostly contributed by contraction of plasma volume possibly due to dehydration. Another seventy-two (72) donors had absolute erythrocytosis. A total of forty-nine (49) donors had background history of chronic cigarette smoking.

We found that there were associations between smoking and thalassaemia/haemoglobinopathy with erythrocytosis in blood donors. We also found positive correlation between the numbers of blood donation with the degree of erythrocytosis ($r = 0.201$). The higher the number of blood donations, the higher Hb level will be observed.

In conclusion, high Hb in blood donors does indicate hidden pathological aetiology. Quite a number of donors were found to have other significant medical conditions such as thalassaemia/haemoglobinopathy and iron deficiency state. We also strongly suggest that blood donors with high Hb level should be investigated thoroughly in order to exclude any significant pathological conditions that are associated with erythrocytosis.

CLINICAL AND LABORATORY PROFILES OF BLOOD DONORS WITH ERYTHROCYTOSIS

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Introduction: In blood banking setting, every blood donors are mandatory to have their haemoglobin (Hb) values tested before blood donation procedure. In current practice, donors with high Hb will be deferred from blood donation. Little attention is given to blood donors with high Hb although it is known that high Hb may indicate an underlying hidden pathological condition.

Objective: The aim of this study is to evaluate the possible underlying aetiologies associated with erythrocytosis in blood donors.

Patients and Methods: A pilot cross sectional study was conducted in Hospital Sultanah Aminah Johor Bahru (HSAJB) and Hospital Universiti Sains Malaysia Kubang Kerian (HUSM) over nine-month period involving blood donors with high pre-donation Hb. A total of 175 samples were collected and analysed for haematological tests, Hb analysis,

serum ferritin, biochemical tests and molecular study for the detection of JAK2 V617F mutation. All of the data were analysed using SPSS software version 23.0.

Results: The prevalence of blood donors with erythrocytosis was 7.8% (n=175). Among 175 donors, 103 donors were found to have relative erythrocytosis, which mostly contributed by contraction of plasma volume possibly due to dehydration. Another seventy-two (72) donors had absolute erythrocytosis. A total of forty-nine (49) donors had background history of chronic cigarette smoking. We found that there were associations between smoking and thalassaemia/ haemoglobinopathy with erythrocytosis in blood donors. We also found positive correlation between the numbers of blood donation with the degree of erythrocytosis ($r = 0.201$). The higher the number of blood donations, the higher Hb level will be observed.

Conclusion: High Hb in blood donors does indicate hidden pathological aetiology. Quite a number of donors were found to have other significant medical conditions such as thalassaemia/haemoglobinopathy and iron deficiency state. We also strongly suggest that blood donors with high Hb level should be investigated thoroughly in order to exclude any significant pathological conditions that are associated with erythrocytosis.

Dr Noor Haslina Mohd Noor: Supervisor

Dr Mohd Nazri Hassan: Co-Supervisor

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Chapter 1

Introduction

1.0 GENERAL INTRODUCTION

Erythrocytosis is defined as an increased in the amount of erythrocytes in the whole blood. It is suspected when the individual presented with a haemoglobin (Hb) or haematocrit (HCT) above the normal reference range (i.e. Hb level is above 18.5 g/dL or the packed cell volume (PCV)/ HCT is greater than 0.52 in male; or 16.5 g/dL and 0.48 in female respectively) (Keohane *et al.*, 2013).

Polycythaemia and erythrocytosis is frequently used synonymously. Traditionally, the term polycythaemia refers to a cluster of disorders, typically characterized by a persistent elevation in HCT due to increased in erythrocytes in the blood circulation (Hoffbrand and Moss, 2011). However, polycythaemia may also be referred to the clonal disorder, such as polycythaemia vera (PV), in which all three haematopoietic cell lineages are involved causing erythrocytosis, frequently with leukocytosis and thrombocytosis (McMullin *et al.*, 2005). When only red cell lineage is affected, thus the term erythrocytosis is more appropriate compared to polycythaemia.

The classification of erythrocytosis depends on the results of a Red Cell Mass (RCM) measurement, in which the results will separate them into two different groups; the absolute erythrocytosis in which both HCT and RCM are above the predicted normal reference range, and the relative erythrocytosis in which the HCT is high but the RCM is in the normal reference range. However, this definition is based on a mutual definition of a raised packed cell volume or HCT and a raised RCM (McMullin, 2008). The possible aetiology for an absolute erythrocytosis can be divided into primary causes, in which there is an intrinsic problem in the bone marrow resulting in increased

erythroid production, or secondary causes; where there is an event outside the bone marrow which accelerates the process of erythropoiesis. The remaining are classified as unexplained group idiopathic erythrocytosis (McMullin, 2008).

Individuals who were found to have high RCM and following investigations do not fulfilled any criteria of primary or secondary erythrocytosis were known as idiopathic erythrocytosis (IE) (Messinezy and Pearson, 1999).

In general, whole blood donation does not cause significant changes in the blood count parameters as blood cells will be replenished quickly by bone marrow approximately around duration of four to eight weeks. For this reason, subsequent donation also will be allowed at least 8 weeks apart from the previous blood donation. Haematinics tablets and advice on iron-rich diet also will be given post-donation to prevent the development of iron deficiency anaemia due to repeated donation especially in regular blood donors. Therefore, blood donors are expected to have normal blood count parameters during subsequent donation.

In blood banking setting, blood donors are mandatory to have their Hb values tested regularly before blood donation procedures in order to exclude anaemia, which is the most frequent cause for temporary deferral. However, it is not uncommon to observe Hb value above or near to the upper limit of normal reference range. In current Malaysian transfusion medicine practices, all blood donors with high Hb (more than 18 g/L) will be deferred from donation.

In current practice, less attention is paid to blood donors with high Hb or at upper limit level of Hb compared to the anaemic donors although it is known that high Hb may be a sign of a disease and a risk factor for vascular accidents. One of the reasons may be due to donors appearing healthy and did not show any sign of diseases.

However, the diagnosis and clinical assessment in such donors may be complicated by frequent, regular blood donations that can mask an underlying pathological disease such as Myeloproliferative Neoplasm (MPN) due to steady reduction of blood mass caused by the donations (Tagariello *et al.*, 2009).

The possibilities of erythrocytosis among blood donor include physiological variation, early phase of Polycythaemia Vera (PV) in which 10-15% will express obvious characteristics of the disease later, unrecognised congenital erythrocytosis, unrecognised secondary acquired erythrocytosis or recently undescribed form of primary or secondary erythrocytosis (Messinezy and Pearson, 1999).

A study done at a hospital in Milan, Italy showed that around 8% of regular volunteer blood donors had high Hb and HCT level. This study also found that some donors with upper limit of Hb and HCT level prove to have underlying acquired pathological condition such as early stage of PV; a type of MPN, as well as respiratory failure. Thus, the study made a conclusion that some donors with upper limit Hb and HCT levels have hidden medical problems (Zanella *et al.*, 1987).

On the other hand, another study done on a group of patients with underlying MPN and found that 18.1% of the patients were or had been blood donors. Interestingly, there was

no other single feature except that blood donation was common in the past history of those patients. Because of that, the study highlighted on the importance of paying due attention to the blood cell count of the donors (Randi *et al.*, 1994).

Thus, it is hoped that this study will contribute as a part of health screening service for blood donors, functioning to detect or diagnose associated medical problems when having high Hb level and also can be considered to be included in the national guidelines on management of blood donors with erythrocytosis.

Since there is no local data available on erythrocytosis in blood donors in Malaysia, it is hoped that the result of this study can be used as one of the referral data for such donors in future.

Chapter 2

Literature Review

2.0 LITERATURE REVIEW

2.1 Blood donation

Blood donation can be categorized into two types namely as whole blood donation and apheresis donation. Whole blood donation means the whole blood is collected from the donor whereas in apheresis donation, only selected blood component is collected. Whole blood constitutes of red blood cells (RBCs), white blood cells (WBCs), platelet, plasma and few other components. It becomes the main source for the preparation of other blood components where the separation involves manual processing procedure under controlled temperature with specific centrifugation speed following the specific component processing guidelines.

On the other hand, the word “apheresis” is originally come from the word “aphairesis”, a Greek word, which means “to separate,” “to take away by force,” or “to remove”. The process started with the withdrawal of whole blood from a donor, extraction of the desired component into a collecting blood bags through a filter membrane or by centrifugation method, and returning the other unused blood component back into the donor. Apheresis also can be used as a therapeutic method in some of the medical cases, act by removing specific pathologic components or substances from a patient’s blood for therapeutic purpose (Winters, 2012).

2.1.1 Donor selection and deferral

Blood and blood component is one of the important treatment modalities and constantly on demand as they are used in many medical procedures. Thus, it is very important to ensure that the blood is safe for usage and come from the safe donors. In most of the countries in the world including Malaysia, blood supply is contributed by voluntary, non-remunerated blood donors. For that safety reason, blood donor criteria selection is created to screen and to ensure the individuals who fulfilled the criteria will only be accepted to donate blood (Eder *et al.*, 2009).

Deferral of the donors who are not fulfilled the selection criteria can occur at any stage of blood donation started from registration, during the blood donation or even after the blood donation. This precautionary measure was taken in order to protect both of the blood donors as well as patients whom received the transfusion. Pre-donation counselling particularly is conducted to assess donor eligibility, complemented by various blood unit testing which will be done later, contribute to the safety of the unit (Sandborg and Thornton, 1994).

Pre-donation interview alone considered as one of the important preliminary measure to capture the possibility of window period for certain communicable diseases, automatically reducing the risk of disease transmission, especially in cases that being undetected by standard blood serology tests (Germain and Goldman, 2002).

Individuals who do not eligible for blood donation will be deferred either temporarily or permanently depending on the cause of deferral. A temporary deferral can be as short as

twenty four hours to as long as two years, whereas permanent deferral refers to a blood donor who disqualified from blood donation indefinitely. The commonly reported cause for temporary deferral are low pre-donation haemoglobin level, low or high blood pressure, underweight, taking medications (e.g. antibiotics), medical conditions (e.g. upper respiratory tract infection), minor or major surgical procedures (e.g. acupuncture, tattoo, dental procedures) (Germain and Goldman, 2002).

The donor deferral rates vary from a blood donation centre to another, estimated around 5% to 25%. In Malaysia, it is estimated around 15% to 20% of blood donors are reported to be deferred from blood donation in Pusat Darah Negara as well as throughout the countries every year. Most of them are temporarily deferred, and the top three listed for this deferral are contributed by low haemoglobin level, followed by high or low blood pressure. About 10% of donor deferrals are permanent deferral in which majority of them are due to high risk behaviour (Seong, 2013).

Studies done in Hospital Universiti Sains Malaysia Kubang Kerian (HUSM) showed around 5.6% blood donors are deferred in this blood centre in year 2006, and the majority of them (64.1%) are regular donors. The study also reported the similar finding for the cause of deferral as Pusat Darah Negara, leads by low haemoglobin level (40.7%) with female donors dominating (69%) in this category. The second common cause are high blood pressure (29.4%), dominated by male donor which constitute around 45%. The other causes include medical illness (15.6%), low blood pressure (3.5%), short interval between donation (1.7%), polycythaemia (1.7%) and other causes of deferral (5.2%) (Rabeya *et al.*, 2008).

A retrospective study done among Turkish population in 5 years starting from 2001 until 2006 according to the gender, age and education level also showed quite identical findings in term of cause of donor deferral; low Hb (20.7%), medical condition such as upper respiratory tract infection (17.7%), hypertension (5.6%), polycythaemia (5.6%) with addition of high risk sex partner (16.7%) as top five listed in the whole group. The leading deferral reason in male donor is upper respiratory tract infection while in female donors is due to low level of haemoglobin. Interestingly, the education level of donors showed no effect on the rate of deferral for both genders at different age group (Arslan, 2007).

An individual who does not meet the blood donor selection criteria (e.g. low haemoglobin, low blood pressure) may experience adverse donor reaction such as headache, giddiness or fainting episode. They will be temporarily deferred with proper advised and encourage to return for blood donation after the deferral period (Halperin *et al.*, 1998).

On the other hand, blood donor will be permanently disqualified from blood donation due to medical conditions or due to their high risk behaviour and lifestyle (high risk groups). Donors with medical conditions such as malignancy, bleeding abnormality as well heart diseases are prohibited from blood donation as this process will potentially affect their well being, thus permanent deferral is indicated. High risk group (e.g. individuals with multiple sex partners, sex workers, and intravenous drug users) will be permanently deferred in view of risk of transmitting transfusion transmitted disease (Lim *et al.*, 1993).

Permanent deferral could affect a donor psychologically, thus the purpose of donor deferral must be clearly explained considering it is the best interest for both blood donor and blood recipient. Understanding this will make it easier for a blood donor to accept the deferral, either temporary or permanent. Permanently deferred donors will be counselled appropriately and professionally by trained staff so that the reason of being permanently deferred can be accepted (Lim *et al.*, 1993).

Blood donor selection criteria are also regularly reviewed to improve the selection criteria and to prevent unnecessary donor deferral. Clearly, this selection guidelines help to avoid or reduce harm to the blood donors, identify any probable significant pathology in the donors, maximizing the therapeutic quality of the ultimate blood product as well as to prevent any damage to the recipient (Arslan, 2007).

2.2 Haematopoiesis

Haematopoiesis is the production of blood cell components. The word haematopoiesis itself is derived from a Greek word meaning "blood" and "to make". In a healthy adult person, large numbers of new blood cells are produced (approximately 10^{11} – 10^{12}) everyday in order to sustain steady state levels in the peripheral circulation. The unique ability of pluri-, multipotent and lineage-committed cells give rise to the formation of different types of mature and functional blood cells via a cycle of proliferation and differentiation pathways. These stem cells will undergo asymmetric division, whereby a precursor cell must divide and expand in every stage of the process before further differentiating along a given pathway in order to maintain the steady-state levels of each cell type. Hematopoietic stem cells which are found in latent state in the bone marrow, forming the basis for this hierarchy and are maintained in steady numbers by self-renewal properties (Durand and Dzierzak, 2005).

Committed progenitor cells will be yielded and accumulated from pluripotent haematopoietic stem cells after a series of division. Although these cells preserve some stem cell-like properties, but their fate is more restricted towards a specific lineage evidenced by the possibility to isolate common lymphoid and myeloid progenitor cells that exhibit specific preferences to the lymphoid and myeloid lineages. Common lymphoid precursors will ultimately develop into T- and B- lymphocytes, whereas common myeloid precursors will differentiated into erythroid cells, megakaryocytes (Meg-E fate) or granulocytes and monocytes/macrophages (GM fate) respectively (Rosmarin *et al.*, 2005). Over years, many studies has reported that development and

differentiation of haematopoietic cells are mediated by cytokines and their receptors (Ogawa, 1993).

Interestingly, recent studies conducted over the past few decades have revealed that Janus Kinases (JAKs), a family of tyrosine kinase and their downstream transcription factors known as STATs (signal transducers and activators of transcription) have been demonstrated to responsible largely in signalling for the hematopoietic cytokine receptor. Therefore, any anomaly in these pathways, such as that is caused by the recently recognized JAK2V617F mutation, becomes an underlying cause for diseases such as leukaemias and other MPN. This recent discovery, when combined with the fact that STATs are activated by oncoproteins such as BCR-ABL, underscores the importance of the JAK-STAT pathway in both normal cellular development and disease states (Jatiani *et al.*, 2010).

2.2.1 Janus Kinases (JAKs) and JAK/STAT pathway

The blood cell production is tightly controlled by a number of growth factors and cytokines that influence the survival, proliferation and differentiation of cells. Most of these molecules attach to the receptors of the cell membrane, which belong to a family of closely related cytokine receptors that lack intrinsic catalytic activity but are closely related with tyrosine kinases of the Janus Kinase (JAK) family (Khwaja, 2006).

Janus kinases were recognized as a unique family of cytoplasmic tyrosine kinase, comprises of four members known as JAK1, JAK2, JAK3 and TYK2. Each of them is composed of seven JAK homology (JH) domains divided into three specific domain, namely as typical kinase (JH1), JAK homology domain 2 (JH2), and regions of homology (JH3-JH7) that are found in the N-terminal half of the protein, which are exclusive among the JAK family (Figure 2.1) (Ihle and Gilliland, 2007).

JH1 domain contains the catalytic activity of tyrosine kinase where as JH2 domain plays an important role as a negative kinase inhibitor on JH1 activity. Hence, any deletion or mutation occurs in JH2 will resulting in constitutive activation of the kinase. The JH2 domain also mediates interaction with STAT family members that are targets of the JAK kinases (Khwaja, 2006).

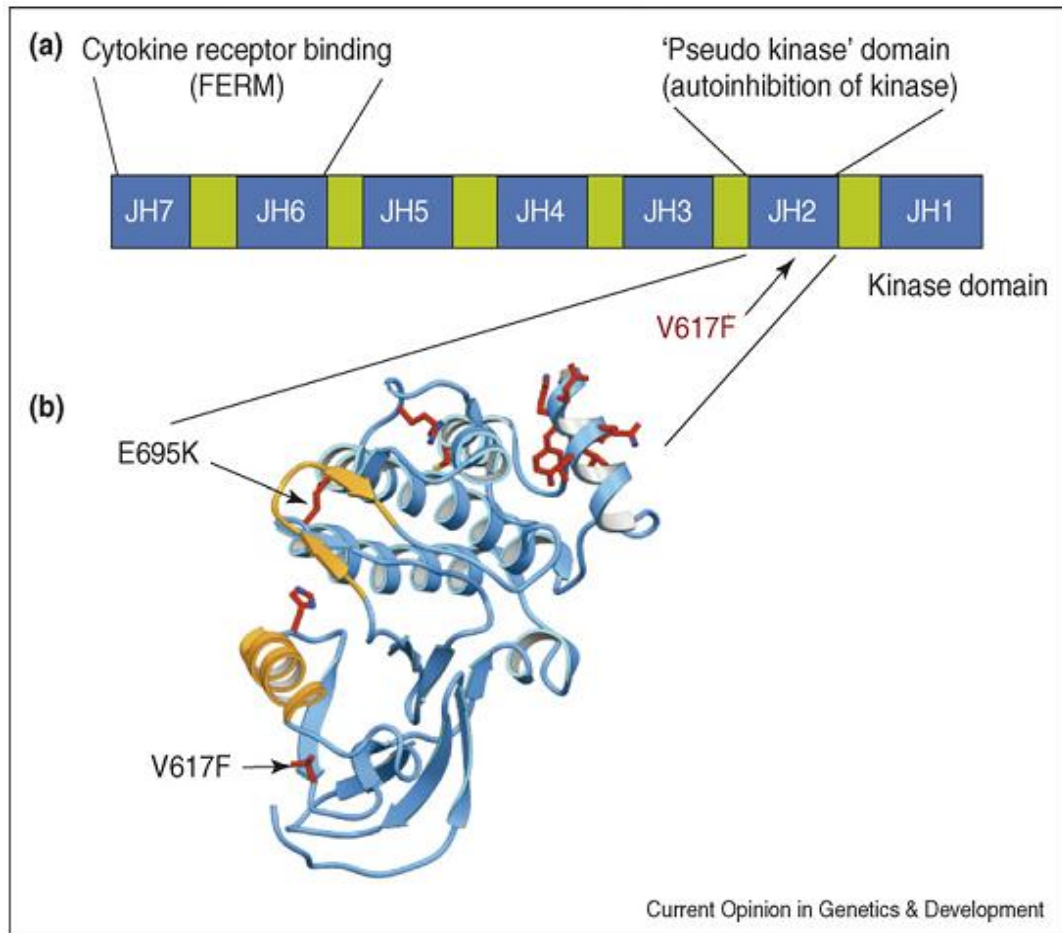


Figure 2.1: Structures of JAK2 and JAKV617F.

(Adapted from (Ihle and Gilliland, 2007).

Like most of tyrosine kinase, the activity of the JAKs is also tightly regulated and phosphorylation process is necessary within the activation loop of the kinase to attain a significant catalytic activity level. At the moment, JAK2 is the only JAK family member acknowledged so far to be implicated in human leukaemia (Valentino and Pierre, 2006).

Transcription factors known as STAT proteins (Signal Transducers and Activators of Transcription) were essential in mediating almost all cytokine driven signalling. These proteins are remaining latent in the cells' cytoplasm and their activation and function are described in Figure 2.2. In normal cells, activation of the STATs via ligand binding is a temporary event whereby in many neoplastic cells, the STAT proteins are persistently activated. (Bromberg and Darnell Jr, 2000).

The JAK2/STAT pathway has a significant responsibility in controlling cell proliferation, activation and apoptosis. Therefore, congenital deficiencies in JAK-STAT signalling are associated with immunodeficiency states whereas any acquired mutations and translocations that causing its activation are involved in the pathophysiology of haematological disorders (Jatiani *et al.*, 2010).

Recently, STAT phosphorylation and its activation are found can be mediated by a number of non-receptor tyrosine kinase such as via oncogenes ; src and abl (Bromberg and Darnell Jr, 2000).

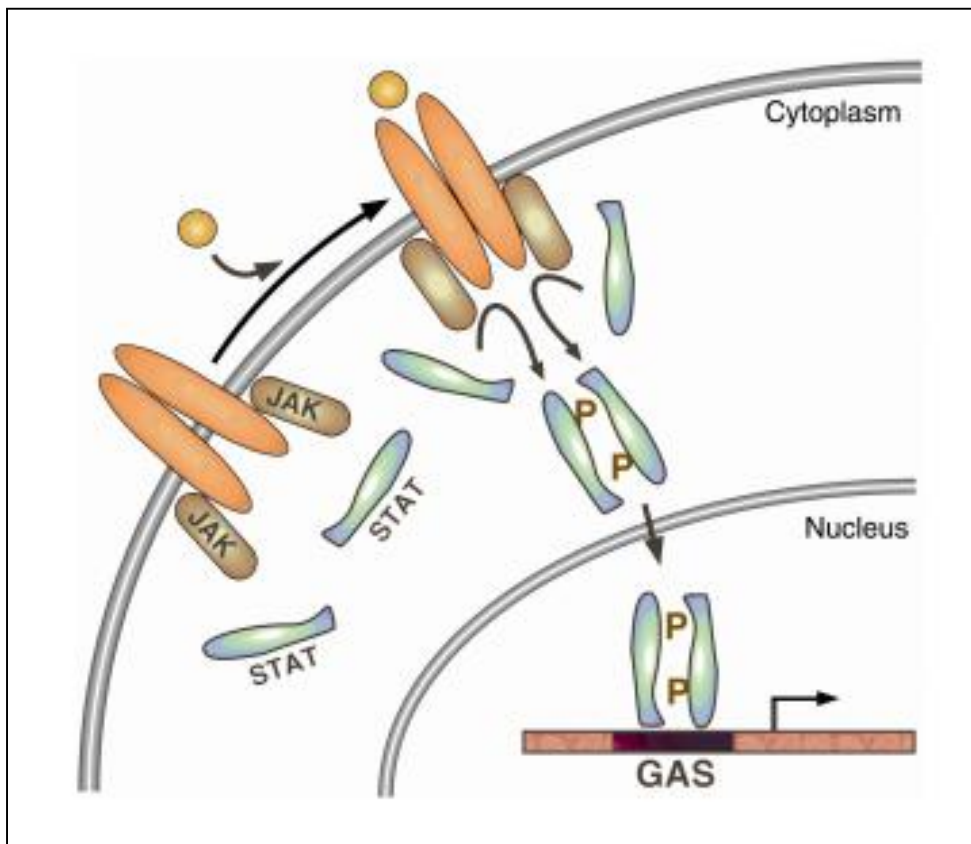


Figure 2.2: The JAK-STAT signalling pathway. Following attachment of a ligand to its receptor, receptor-associated JAKs become and mediated phosphorylation of specific receptor tyrosine-kinase residues. This leads to the recruitment of specific STATs which are then also tyrosine phosphorylated. Activated STATs are released from the receptor, undergo dimerization and translocate to the nucleus, finally bind to specific regulatory sequences to activate or repress the transcription of target genes. Thus, the JAK/STAT cascade provides a direct mechanism for translating an extracellular signal into a transcriptional response.

(Adapted from (Schindler, 2002)).

2.2.2 JAK2 V617 mutation

JAK2 gene is one of the member of the Janus kinase family. It can be found on chromosome 9, encodes for JAK2 protein. The normal JAK2 protein is a cytoplasmic tyrosine kinase, which associated with cytoplasmic domain of haematopoietic growth factors and cytokines such as erythropoietin, thromboietin, interleukin-3 (IL-3), granulocytes colony stimulating factors (G-CSF) and granulocyte macrophage colony stimulating factors. In other word, it functions as an intermediate between membrane receptors and signalling molecules in the cell signalling pathway. When these molecules bind to their respective receptors, dimerization of the subunits occurs, causing the JAK2 are brought in proximity and undergo transphosphorylation causing its activation (Baxter *et al.*, 2005).

Clonal, acquired point mutation of JAK2 gene, leads to substitution of Valine-to-Phenylalanine at amino acid 617 (V617F) due to replacement of Guanine (G) by Thymine (T) in the nucleotide sequences (1848 G > T), causing constitutive activation of the tyrosine kinase that is believed to confer erythropoietin-independent hypersensitivity and erythropoietin-independent survival to the myeloid-stem cell. Thus, mutation in the JAK2 causing that this kinase remain active even without the growth factors stimulation resulting in continuous proliferation of mature cells. Many studies show JAK2 mutation has been found in the majority of patients with PV and also in other MPN. A point mutation in the JAK2 gene was identified in several MPN, most frequently in Polycythaemia Vera (65-97%), Essential Thrombocythaemia (23-57%) and Myelofibrosis (35-57%). It is also occasionally present in Myelodysplastic

Syndrome, Chronic Myelomonocytic Leukaemia and other atypical myeloid disorders (Ihle and Gilliland, 2007).

Some studies demonstrated that JAK2 mutation is more common than MPN and can be found in other patients with non-haematological disorders. Interestingly, most of the JAK2 mutation carriers have the same finding on blood cell parameters; normal red cell count but significantly higher in leukocytes and platelet counts although most were within the normal reference limit. Its presence may be useful for the early identification of haematological disorders but may not be used exclusively for the diagnosis of MPN (Xu *et al.*, 2007).

Detection of JAK2 mutation also at very low level in healthy individuals who have perfectly normal blood count is also reported in few studies. Thus, the conclusion made that this type of mutation is probably unrelated with the progression of the disease but its presence may indicate that a very early molecular event has occurred prior to the manifestation of haematological disorders, especially MPN phenotype. This mutational event itself may not be adequate enough to provoke MPN. This study data was supported by the finding of low annual incidence of MPN reported compared to the frequency of individuals harbouring JAK2 mutation (Sidon *et al.*, 2006).

Interestingly, the prevalence and the frequency of JAK2 mutation also was found higher in smokers compared to non smokers population, contributed by the acceleration of erythropoiesis which causes the haematopoietic cells susceptible to JAK2 mutation. However, there is no correlation that can be concluded between the prevalence of the JAK2

mutation or the frequency of the mutation with RBC, HCT or WBC count (Weinberg *et al.*, 2012).

2.3 Erythrocytosis

Erythrocytosis encompasses a number of disorders characterized by increased circulating red blood cells (RBCs) which can be classified into relative, absolute and idiopathic erythrocytosis (McMullin, 2008).

Relative erythrocytosis refers to a condition in which the HCT is elevated, normal RCM, while the plasma volume may be reduced. It is associated with various events such as acute hypoxia, cigarette smoking, excessive alcohol intake and the usage of diuretics (Biswas *et al.*, 2003).

Absolute erythrocytosis can further divided into primary and secondary forms (Table 2.1). Primary erythrocytosis are characterized by expanding of erythropoietic compartment independent of erythropoietin (EPO) and/or abnormally increased in the response of the haematopoietic precursors to EPO due to hereditary or acquired somatic mutations. On the other hand, in secondary erythrocytosis, the erythroid precursors show normal responsiveness towards erythropoietin, but there are increased in the levels of circulating factors driving erythropoiesis (most commonly erythropoietin, insulin growth factor I, angiotensin II/angiotensin receptor axis aberrations and cobalt) (Finazzi *et al.*, 2006).

Secondary erythrocytosis may come up from several causes including inappropriate erythropoietin production, renal tumours and other kidney diseases, but association with defective oxygen transport is rare and usually caused by abnormal Hb with increased

oxygen affinity. Most patients with this condition appear in good health but had higher than normal RBC and Hb levels in the blood (Prakobkaew *et al.*, 2010).

Secondary erythrocytosis associated with thalassaemias and haemoglobinopathies is also rare, and among those which produce erythrocytosis, only moderate degrees of elevation of Hb and RBC counts have generally been found (Bessman, 1977; Fairbanks *et al.*, 1979).

Idiopathic erythrocytosis (IE) constitutes the remaining group of individuals in whom the cause for absolute erythrocytosis has not yet been identified. In other words, it is defined as an increased in RCM without an identified cause, i.e. the underlying cause for acceleration in erythropoiesis is unknown. Its diagnosis is based on the exclusion of various primary or secondary polycythaemias (congenital or acquired). Heterogenous mechanisms underlying IE have been suggested, including early stage of PV and unrecognized congenital or secondary polycythaemia (Messinezy and Pearson, 1999).

Table 2.1: Pathophysiological classification of absolute erythrocytosis.

Primary polycythaemia:
Congenital
• Primary familial congenital polycythaemia (including mutations of the EPO receptor)
Acquired
• Polycythaemia Vera
Secondary erythrocytosis:
Congenital
• Mutant high oxygen-affinity haemoglobins
• Congenital low 2,3-biphosphoglycerate deficiency
• Methaemoglobinaemia
• Chuvash polycythaemia and other VHL mutations (with some features of primary polycythaemia) (autonomus high EPO production)
Acquired
• Hypoxaemia (chronic lung disease, high altitude, cyanotic congenital heart disease)
• Renal disease (tumours, cysts, hydronephrosis, renal artery stenosis, renal transplantation)
• Tumours (cerebellar haemangioblastoma, phaeochromocytoma, paraganglioma, uterine fibroids)
• Drugs (erythropoietin, androgens)
Idiopathic erythrocytosis

(Adapted from (McMullin, 2008).

2.4 Primary erythrocytosis

Primary erythrocytosis is applied when there is an intrinsic defect of the erythroid component in the bone marrow. It can be classified into congenital and acquired cause. However, the major cause of primary erythrocytosis is Polycythaemia Vera (PV) in which the marrow producing too many erythrocytes, and commonly with increased in granulocytes as well as platelets (McMullin, 2008).

2.4.1 Congenital causes

Erythrocytosis due to congenital causes are those in which an underlying genetic abnormality has been recognized. The usual presentation is at a younger age as the mutation has been exist since birth with positive family history of erythrocytosis as the defect is inherited although possibility of new mutations can arise. Mutation of erythropoietin receptor (EPOR) gene is rare but presented as one of the causes that can give rise to congenital erythrocytosis (Percy *et al.*, 1998).

Physiologically, the hormone EPO binds to its receptor on the cell surface, and initiates a series of events in which other proteins are recruited, phosphorylated and translocated to the nucleus, leads to gene transcription where a further signal results in increased erythropoiesis. Inhibition of this process will take place whenever sufficient erythrocytes have been produced, mediated by the attachment of the SHP-1 protein to the EPOR, which dephosphorylates the receptors (de La Chapelle *et al.*, 1993).